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Hymenochaete in China. 4. *H. parmastoi* sp. nov. and *H. ustulata* new to China

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Abstract — *Hymenochaete parmastoi* is described as new to science. It is similar to *H. minor* and *H. rhabarbarina*, but morphological and molecular (ITS rDNA sequences) evidence show that they are distinct from each other. *Hymenochaete ustulata*, which was collected from Guangdong Province, southern China, is newly recorded in China. It is characterized by anatomical structure (in sect. *Paragymnochaete* and with abundant crystals). Illustrated descriptions of these two species are provided.

Key words — *Hymenochaetaceae*, phylogeny, taxonomy, wood-inhabiting fungi

Introduction

The poroid wood-inhabiting fungi in China have been intensively studied during the last decade (Yuan & Dai 2008a,b; Dai 2010, 2011, 2012; Cui et al. 2011; Dai & Cui 2011; Zhou & Dai 2012), but the corticioid fungi are still poorly studied. As a contribution to the studies of the corticioid fungi in China, a project dealing with the taxonomy and phylogeny of the genus *Hymenochaete* Lév. (Léveillé 1846) is in progress and several intensive surveys in south and southwest China have been carried out since 2010. Among the 1000 specimens collected, several new species and Chinese new records have been found (He & Li 2011a,b, 2012a,b, He & Dai 2012).

More recently, careful morphological study and molecular analysis of some *Hymenochaete* specimens from several provinces and autonomous regions in temperate and subtropical areas of China indicate that they represent an undescribed species, which we describe here as *H. parmastoi*.

One additional specimen collected from Guangdong Province is identified as *H. ustulata*, a rare species new to Chinese fungal flora. This is the first report outside its type locality. Illustrated descriptions of these two species are provided in this paper.

Materials & methods

Morphological studies: Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure follows Dai (2010). In presenting the size range of spores and setae, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetical average of all spores), W = mean spore width (arithmetical average of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = the number of spores measured from given number of specimens. IKI stands for Melzer's reagent, KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue in lactic acid. IKI– = inamyloid and nondextrinoid, CB– = acyanophilous. Special color terms follow Petersen (1996).

DNA extraction and sequencing: The F-130 Phire® Plant Direct PCR Kit (Finnzymes, Finland) was employed for DNA extraction and PCR amplification from herbarium specimens according to the manufacturer's instructions. Approximately 700 base pairs of the ITS rDNA were amplified with primers ITS5 (gga agt aaa agt cgt aac aag g) and ITS4 (tcc tcc gct tat tga tat gc; White et al. 1990), using the following procedure: initial denaturation at 98°C for 5 min, followed by 39 cycles of 98°C for 5 s, 58°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and all new sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis: The phylogeny was inferred from the ITS sequences of 51 *Hymenochaete* species. *Pseudochaete corrugata* (Fr.) S.H. He & Y.C. Dai and *P. tabacina* (Sowerby) T. Wagner & M. Fisch. were selected as outgroups. The sequences were aligned using the ClustalX 1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit 7.0.5.3 (Hall 1999). Maximum parsimony analysis were performed using PAUP* 4.0b10 (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analysis were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each tree generated. Trees were figured in Treeview 1.6.6 (Page 1996).

Taxonomy

Hymenochaete parmastoi S.H. He & Hai J. Li, **sp. nov.** Figs 1–2

MycoBank MB 800487

Differs from other *Hymenochaete* species by its yellowish brown basidiocarps, encrusted setae, and ellipsoid to ovoid basidiospores.

Type: China. Xizang Autonomous Region, Bomi County, on fallen angiosperm trunk, 20.IX.2010, He 367 (**holotype**, BJFC; GenBank, JQ780061).

Fig. 1. Basidiocarps of *Hymenochaete parmastoi* (holotype).

Etymology: honoring Erast Parmasto, 1928–2012, eminent Estonian mycologist and author of many significant papers on *Hymenochaete*.

Fruitbody: Basidiocarps annual, effused, closely adnate, coriaceous, first as small round or irregular colonies, later confluent up to 15 cm or more in longest dimension, 50–250 μm thick in section. Hymenophore smooth, cinnamon to clay-buff, not cracked or with numerous deep crevices with age; margin thinning out, distinct, whitish to yellowish when juvenile, becoming indistinct, concolorous with hymenophore surface when mature.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Tomentum and cortex absent. Hyphal layer locally present, thin. Generative hyphae hyaline to yellowish brown, thin- to thick-walled with a wide lumen, septate, usually branched at a right angle, loosely to densely interwoven, 2–4 μm in diam.

Stratified hymenium: Hyphae in this layer similar to those in subiculum, yellowish brown, thick-walled, more or less agglutinated, interwoven, 2–3 μm in diam. Setal layer composed of 1–3 rows of overlapping setae. Setae not numerous, scattered, subulate, reddish brown, with acute tips, projecting up to 30 μm above the hymenium, (30–)40–75(–95) × (5–)6–10 μm, usually enmeshed in thick hyphal sheath, this usually slightly or heavily encrusted with yellowish or brownish amorphous matters in the tip part. Cystidia and hyphidia absent, but hyphal ends sometimes present in the hymenium, some encrusted

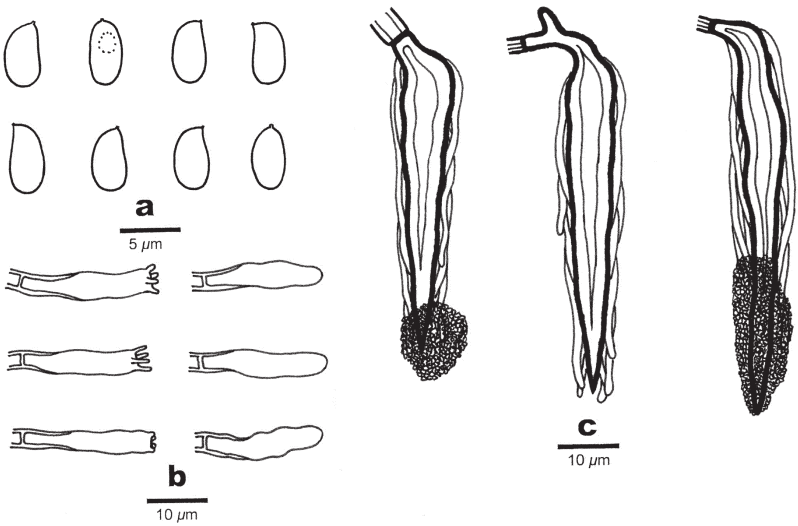

Fig. 2. Microscopic structures of *Hymenochaete parmastoi* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Setae.

with amorphous matters. Basidia clavate, with four sterigmata and a simple septum at base, walls thickening at basal part, 15–20 × 3–4.8 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores: Basidiospores ellipsoid to ovoid, hyaline, thin-walled, smooth, IKI–, CB–, (3.8–)4–5.5(–6) × (2.5–)2.6–3.2(–3.5) μm, L = 4.84 μm, W = 2.94 μm, Q = 1.55–1.76 (n = 210/7).

Additional specimens examined: **China**. **Anhui Prov.**, Huangshan County, Yellow Mountain National Park, on fallen twig of *Rhododendron*, 20.X.2010, He 431 (BJFC); **Guangxi Autonomous Region**, Xing'an County, Mao'ershan Nat. Res., on fallen angiosperm twig, 19.VIII.2011, He 867 (BJFC; GenBank, JQ780063); **Guizhou Prov.**, Leishan County, Leigongshan Nat. Res., on fallen angiosperm twig, 24.VIII.2010, He 266 (BJFC), He 277 (BJFC; GenBank, JQ780064); **Xizang Autonomous Region**, Linzhi County, Lulang, on fallen angiosperm twig, 24.IX.2010, He 388 (BJFC; GenBank, JQ780062), He 391 (BJFC); Gadinggou, on fallen angiosperm twig, 25.IX.2010, He 402 (BJFC); **Yunnan Prov.**, Zhanyi County, Zhujiangyuan Forest Park, on dead angiosperm tree, 11.VI.2011, He 725 (BJFC); Chuxiong, Zixishan Forest Park, on fallen angiosperm trunk, 11.VI.2011, He 741 (BJFC).

Hymenochaete ustulata G.A. Escobar ex J.C. Léger, Cryptog. Mycol. 11: 309, 1990 Figs 3–4

Fruitbody: Basidiocarps annual, effused, closely adnate, coriaceous, first as small colonies, later confluent up to 5 cm or more in longest dimension, 100–150

Fig. 3. Basidiocarps of *Hymenochaete ustulata* (He 104).

μm thick in section. Hymenophore smooth or with scattered small tubercles, vinaceous brown to reddish brown when fresh, becoming pale mouse-gray to vinaceous gray after dry, usually not cracked; margin thinning out, indistinct, paler or concolorous with hymenophore surface.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Tomentum and hyphal layer absent. Cortex well developed, composed of strongly agglutinated hyphae, 20–70 μm thick.

Stratified hymenium: Generative hyphae yellowish brown, thick-walled, agglutinated, 2–3.5 μm in diam. Setal layer thickening, composed of several rows of overlapping setae. Agglomerates of crystals frequently present in the setal layer and subhymenium, 10–30(–50) μm in diam. Setae numerous, subulate, reddish brown, with acute tips, some enmeshed with a thin hyphal sheath, projecting up to 40 μm above the hymenium, (30–)45–70(–75) × 5–9 μm. Cystidia and hyphidia absent. Basidia clavate, with four sterigmata and a simple septum at base, walls thickening at basal part, 15–18 × 3–4 μm; basidioles in shape similar to basidia, but smaller.

Spores: Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, 4–5 × 2.2–2.8 μm, L = 4.42 μm, W = 2.49 μm, Q = 1.78 (n = 30/1).

Specimen examined: **CHINA. Guangdong Prov.**, Shixing County, Chebaling Nat. Res., on rotten angiosperm trunk, 24.VI.2010, He 104 (BJFC; GenBank, JQ780066).

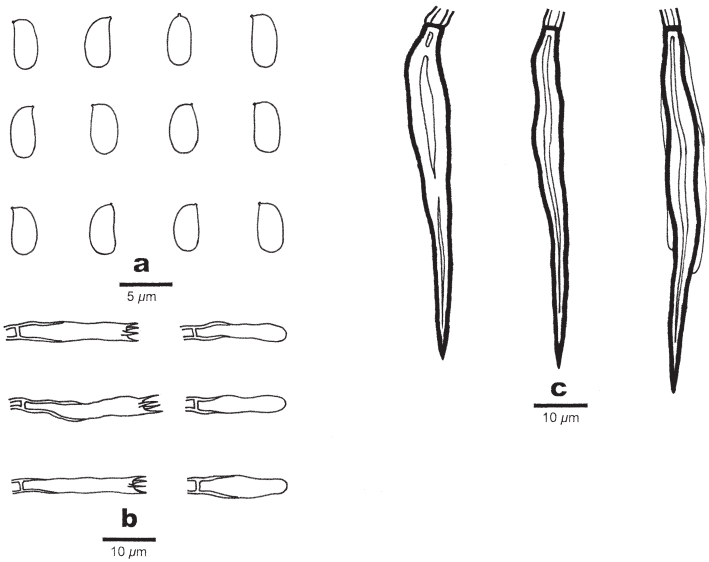

Fig. 4. Microscopic structures of *Hymenochaete ustulata* (drawn from He 104). a: Basidiospores. b: Basidia and basidioles. c: Setae.

Phylogenetic results

Seven ITS sequences were newly obtained in this study (GenBank accession numbers JQ780061–JQ780067). The alignment of the ITS sequences of 53 taxa resulted in 786 sites with 398 parsimony informative characters. One strict consensus tree was yielded from the analysis with TL = 2056, CI = 0.428, RI = 0.645, RC = 0.276 and HI = 0.572 (Fig. 5). The strict consensus tree placed *Hymenochaete* taxa well apart from the outgroups, with *H. parmastoi* strongly supported (bootstrap value = 100%) as a separate lineage and confirming *H. parmastoi* as phylogenetically distinct from any sampled species. The tree also showed *H. parmastoi* closely related to *H. minor* S.H. He & Y.C. Dai and *H. ustulata* closely related to *H. rhabarbarina* (Berk.) Cooke.

Discussion

Hymenochaete parmastoi is characterized by yellowish brown basidiocarps, encrusted setae, and ellipsoid to ovoid basidiospores. In both morphological and phylogenetic aspects, *H. parmastoi* seems very close to *H. minor* (with a 100% bootstrap value), which also has setae encrusted with amorphous matter. However, *H. minor* differs from *H. parmastoi* by its shorter setae (25–50 μm) and smaller basidiospores (3–4.5 × 1.7–2 μm; He & Dai 2012). Moreover, *H. parmastoi* grows mostly in temperate areas, while *H. minor* seems to be a tropical species.

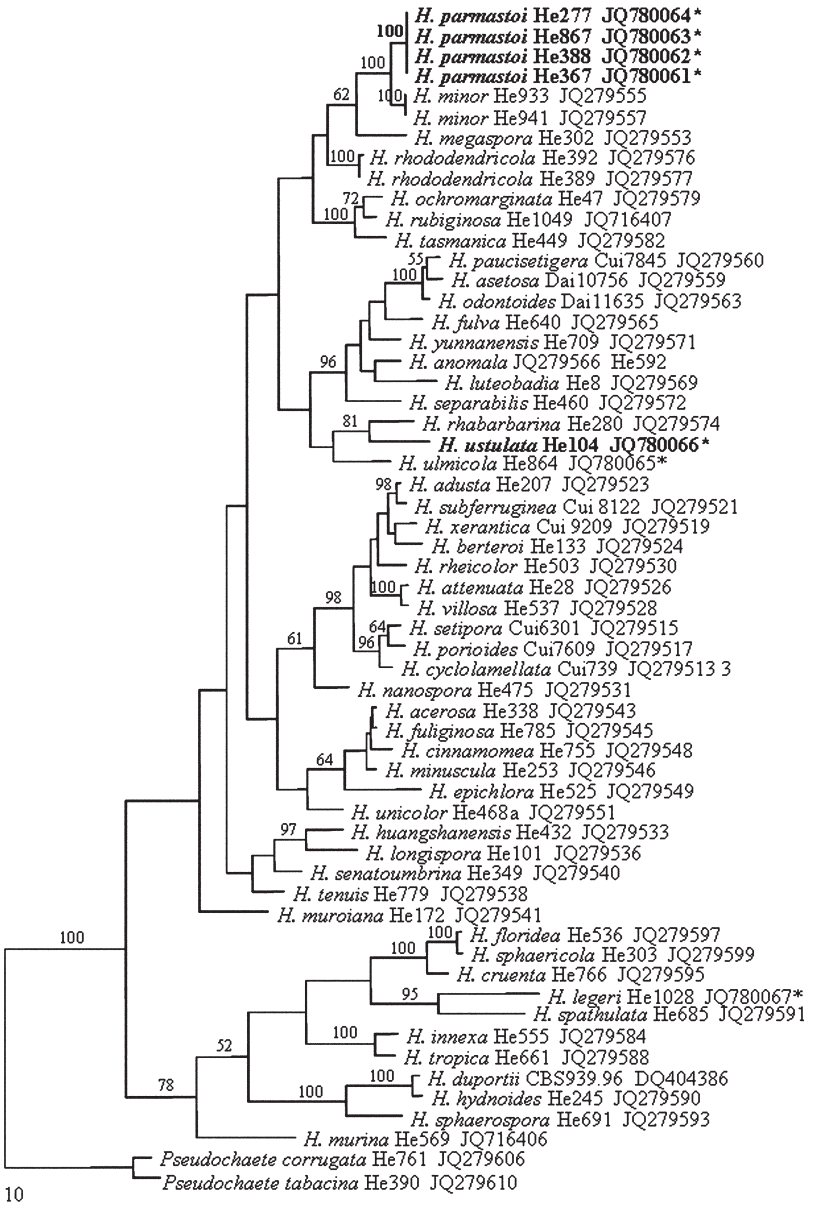
H. parmastoi He277 JQ780064*
H. parmastoi He867 JQ780063*
H. parmastoi He388 JQ780062*
H. parmastoi He367 JQ780061*
H. minor He933 JQ279555
H. minor He941 JQ279557
H. megaspora He302 JQ279553
H. rhododendricola He392 JQ279576
H. rhododendricola He389 JQ279577
H. ochromarginata He47 JQ279579
H. rubiginosa He1049 JQ716407
H. tasmanica He449 JQ279582
H. paucisetigera Cui7845 JQ279560
H. asetosa Dai10756 JQ279559
H. odontoides Dai11635 JQ279563
H. fulva He640 JQ279565
H. yunnanensis He709 JQ279571
H. anomala JQ279566 He592
H. luteobadia He8 JQ279569
H. separabilis He460 JQ279572
H. rhabarbarina He280 JQ279574
H. ustulata He104 JQ780066*
H. ulmicola He864 JQ780065*
H. adusta He207 JQ279523
H. subferruginea Cui 8122 JQ279521
H. xerantica Cui 9209 JQ279519
H. berteroi He133 JQ279524
H. rheicolor He503 JQ279530
H. attenuata He28 JQ279526
H. villosa He537 JQ279528
H. setipora Cui6301 JQ279515
H. porioides Cui7609 JQ279517
H. cyclolamellata Cui739 JQ279513 3
H. nanospora He475 JQ279531
H. acerosa He338 JQ279543
H. fuliginosa He785 JQ279545
H. cinnamomea He755 JQ279548
H. minuscula He253 JQ279546
H. epichlora He525 JQ279549
H. unicolor He468a JQ279551
H. huangshanensis He432 JQ279533
H. longispora He101 JQ279536
H. senatoumbrina He349 JQ279540
H. tenuis He779 JQ279538
H. muroiana He172 JQ279541
H. floridea He536 JQ279597
H. sphaericola He303 JQ279599
H. cruenta He766 JQ279595
H. legeri He1028 JQ780067*
H. spathulata He685 JQ279591
H. innexa He555 JQ279584
H. tropica He661 JQ279588
H. duportii CBS939.96 DQ404386
H. hydnoides He245 JQ279590
H. sphaerospora He691 JQ279593
H. murina He569 JQ716406
Pseudochaete corrugata He761 JQ279606
Pseudochaete tabacina He390 JQ279610
10

FIG. 5. Strict consensus tree obtained from Maximum Parsimony analysis of ITS sequences of *Hymenochaete*. *Pseudochaete corrugata* and *P. tabacina* were used as outgroups. Newly generated sequences are those with *. Parsimony bootstrap values (>50%) are shown.

Two other species, *H. rhabarbarina* and *H. rhododendricola* S.H. He & Hai J. Li, also have setae encrusted with amorphous matter. However, *H. rhabarbarina* differs from *H. parmastoi* by its well-developed hyphal layer, encrusted hyphae, and longer setae (60–100 µm; Parmasto 2001), while *H. rhododendricola* has longer setae (80–130 µm) and larger basidiospores (6–7 × 4–5 µm; He & Li 2011a) than *H. parmastoi*. These two morphologically close allies are distantly related to the *H. parmastoi* clade in the phylogram.

Hymenochaete ustulata was previously reported only in Brazil. The species is diagnosed by its anatomical structure, which contains a well-developed cortex without a hyphal layer (sect. *Paragymnochaete*) and an abundance of crystal agglomerates. The similar *H. separata* G. Cunn. can be separated by its smaller setae (35–45 × 6–7 µm) and larger basidiospores (5–6.5 × 3–3.5 µm; Escobar 1978; Parmasto & Gilbertson 2005). *Hymenochaete dissimilis* G. Cunn. shares similar setae, basidiospores, and agglomerated crystals but differs from *H. ustulata* in its thicker basidiocarps (≤750 µm) and lack of cortex (Cunningham 1957; Léger 1998).

He & Dai (2012) conducted a taxonomic and phylogenetic study of *Hymenochaete* and allied genera in China in which they combined five species of *Hydnochaete* Bres. and *Cyclomyces* Kunze ex Fr. in *Hymenochaete* and transferred five species of *Hymenochaete* were transferred to *Pseudochaete* T. Wagner & M. Fisch. (nom. illegit., non *Pseudochaete* West & G.S. West [*Chlorophyta*]). Including the two species reported in this paper, a total of 55 *Hymenochaete* species have been recorded in China (He & Dai 2012; He & Li 2012b).

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